PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, C07K 14/705, C12Q 1/68, C12N 5/10 — A1

(11) International Publication Number: WO 98/24895

(43) International Publication Date: 11 June 1998 (11.06.98)

(21) International Application Number: PCT/EP97/06252

(22) International Filing Date: 8 November 1997 (08.11.97)

(30) Priority Data:
9625074.1 2 December 1996 (02.12.96) GB

(71) Applicant *(for all designated States except US)*: PHARMACIA & UPJOHN S.P.A. [IT/IT]; Via Robert Koch, 1.2, I–20152 Milano (IT).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: RICCARDI, Carlo [IT/IT]; Via del Favarone, 37, I–06122 Perugia (IT).

(81) Designated States: AU, BR, CA, CN, HU, IL, JP, KR, MX, NO, NZ, PL, SG, UA, US, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: RECEPTOR BELONGING TO THE TNF/NGF RECEPTOR FAMILY

(57) Abstract

The present invention relates to a previously unknown receptor protein, named GITR (Glucocorticoid Induced TNFR–family Related protein), which shares structural and biological characteristics with other members of the tumor necrosis factor/nerve growth factor receptor (TNFR/NGFR) family. Also disclosed are splicing variants of GITR. The invention further relates to polynucleotide sequences encoding the GITR receptor and its splicing variants, vectors comprising such polynucleotide sequences, host cells transformed with the said vector and a recombinant process for producing the proteins of the invention.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Title:

RECEPTOR BELONGING TO THE TNF/NGF RECEPTOR FAMILY

The present invention relates to a previously unknown receptor protein, named GITR (Glucocorticoid Induced TNFR-family Related protein) and previously coded D4, which shares structural and biological characteristics with other members of the tumor necrosis factor/nerve growth factor receptor (TNFR/NGFR) family. Also disclosed are splicing variants of GITR.

BACKGROUND OF THE INVENTION

The proteins belonging to the tumor necrosis factor/nerve growth factor receptor (TNFR/NGFR) family play a crucial role in cell activation, differentiation and death. The signals initiated with the triggering of these receptors by a corresponding family of structurally related ligands, are required for the normal development and function of the immune system. Excessive signaling through some of the receptors can cause severe inflammatory reaction, tissue injury and shock. Mutation of genes corresponding to the ligands or to the receptors can cause characteristic disturbances of lymphocytes, derangement of the immune response or autoimmune disease.

From the structural point of view, members of the TNFR/NGFR family are classified as type I transmembrane proteins characterized in the extracellular portion by the presence of 3-5 similar motifs (the cystein pseudorepeats C-x(4,6)-[FYH]-x(5,10)-C-x(0,2)-C-x(2,3)-C-

x(7,11)-C-x(4,6)-[DNEQSKP]-x(2)-C), which have recently been recognized as true domains (Banner D.W., D'Arcy A., Janes W., Gentz R., Schoenfeld H-J., Broger C., Loetscher H. & Lesslauer W. (1993) Cell 73, 431-445).

Both ligand and receptor are transmembrane proteins (with the only exception of TNF and lymphotoxin-α) and the receptor/ligand interaction takes place following cell-to-cell contact. Each member of the family binds to one specific receptor (with the only exception of TNF and lymphotoxin-α and TRAIL). Not only the receptors but also at least some ligands (e.g. CD40L) transduce intracellular messages (and thus the receptor-ligand distinction blurs).

Schematically, we can consider 2 subfamilies within the TNFR/NGFR family: the receptors mainly inducing apoptosis (TNFR-p55, Fas, CAR1, DR3 and the TRAIL receptors family) and the receptors mainly stimulating cell proliferation, differentiation and activation (TNFR-p75, CD40, CD30, CD27, 4-1BB and OX40). Furthermore, some receptors (e.g. CD40) inhibit cell death.

The apoptosis inducing receptors contain a 60-residue cytoplasm sequence known as the "death domain", required for the transduction of an apoptotic signal. Humans with mutations of the Fas gene have lymphadenopathy, splenomegaly and signs of autoimmunity at an early age. Two poxvirus gene products (T2 and A53R) have been shown to encode soluble, secreted forms of TNFR. These TNFR-like proteins form a complex with (and thereby inactive) host-produced TNF. The extraordinary virulence of wild-type myxoma poxvirus, uniformly fatal to its host (rabbits), is reduced nearly 50% in

recombinants differing only by an inactivated T2 gene. (Upton C., Macen J., Schreiber M., McFaden G. (1991) Virology **184**, 370-382). TRAIL-R3 lacks the splicing domain and it has been recently suggested that TRAIL-R3b expression protects normal cells from TRAIL induced apoptosis (Trisha Gura (1997) Science 277, 768).

The members of TNFR/NGFR family which activate cell function, have specific, non-overlapping role in the maturation of B and T-cells. The syndrome of X-linked immunodeficiency (high levels of IgM and low or absent levels of other immunoglobulins) is caused by a mutation in the CD40L. CD30 was originally described as a marker in the Hodgkin's lymphoma, because overexpressed in these cancerous cells (Schwab U., Stein H, Gerdes J, Lemke H, Kirchner H, Schaadt M & Diehl V (1982) Nature **299**: 65-68). Disregulation of CD30/CD30L interaction was recently demonstrated in atopy, Omenn's syndrome and systemic lupus erythematosus (Del Prete G., Maggi E, Pizzolo G, Romagnani S (1995) Immunol.today **16**(2): 76-80). Moreover, CD30 triggering is involved in promoting HIV replication (Del Prete G., Maggi E, Pizzolo G, Romagnani S (1995) Immunol. today **16**(2): 76-80).

CD40/CD40L, CD27/CD70 and 4-1BB/4-1BBL interactions costimulate T-cells activated through the T cell receptor (TCR)/CD3 complex in a way similar to CD28/CD80 interaction.

The receptors are induced following antigenic stimulation and play a role during T-cell activation.

It appears therefore evident that these receptor proteins are involved in many diseases. Different strategies can be used in different diseases. Stimulation of these

receptors can be useful when lymphocyte activation is needed (e.g., in oncologic patients). Inhibition of these receptors can be helpful when a decrease of lymphocyte reactivity is needed (e.g. autoimmune diseases). Finally, when tumor cells overexpress one of these receptors, this can be used as a tool to target these cells (e.g. by immunotoxins), to inhibit tumor cell proliferation and/or to monitor the response to chemotherapy (absence of minimal residual disease and early diagnosis of relapse). There is therefore the need to gain a deeper insight into the biological mechanisms regulated by or involving the TNFR/NGFR family.

In this context, the inventor of the present invention has now identified a new member of the TNFR/NGFR family, designated GITR, and its splicing variants GITR-B and GITR-C. With the term "splicing variant" it is meant each of the different forms of the GITR receptor deriving from the alternative splicing of the primary transcript. As is well known to the expert in the art, the genomic DNA of eukaryotes is organized in regions called exons and regions called introns. The genomic DNA is transcribed into a primary transcript (nuclear mRNA) containing exons and introns. Introns are subsequently excised and the coding sequences are simultaneously linked by a splicing complex to form the mature mRNA (cytoplasmic mRNA). The organization of the genomic DNA into exons and introns offers the potentiality for generating a series of related proteins by splicing a nascent RNA transcript in different ways. This process is known as alternative splicing and it is a means of forming a set of proteins that are variants of a basic motif. In view of the

structural and biological characteristics of the new receptor, which will be better described below, the protein of the invention find a useful application in several diagnostic as well as therapeutic fields.

In one aspect the protein of the invention can be used as a probe to isolate ligands to GITR.

Taking into account the biological properties of the receptor family to which GITR belongs and the functional knowledge of the protein itself provided herewith, GITR can be used for lymphocyte activity stimulation and cell death rescue. These goals are accomplished by a variety of means: a fusion protein comprising the extracellular portion of GITR could be used to trigger the corresponding ligand; alternatively GITR can be transfected through viral vectors or encapsulated plasmids in unresponsive or low-level responsive T-lymphocytes (such as tumor infiltrating T-lymphocytes); lymphocytes can be treated with agonist antibodies or with a peptide mimicking the intracytoplasmic domain of GITR thus activating the intracellular pathways physiologically initiated by triggering the receptor.

In other circumstances it can be desirable to suppress the lymphocyte activity and to induce apoptotic deletion. This goal can be accomplished by preparing a soluble fusion protein of GITR acting as a decoy target or by treating the lymphocytes with antagonist antibodies or with an antisense oligonucleotide aimed to suppress the expression of GITR; alternatively a mutated GITR construct can be transfected through viral vectors or encapsulated plasmids to act as cell linked decoy target; furthermore, lymphocytes can be treated with a peptide

binding the intracytoplasmic domain of GITR (and possibly 4-1BB and CD27) thus inhibiting the intracellular pathways physiologically initiated by triggering these receptors.

In a further useful application GITR can be used to suppress the growth of tumor cells overexpressing GITR: in this case, approaches similar to those described above to suppress the lymphocyte activity can be followed; moreover, GITR antibodies conjugated with toxins (such as ricin, saporin, momordin) can be used as immunotoxins which target specifically GITR overexpressing tumor cells. A similar approach has been used successfully to cure SCID mice with human xenografted CD30+ anaplastic large-cell lymphoma (Pasqualucci L., Wasik M., Teicher B.A., Flenghi L., Bolognesi A., Stirpe F., Polito L., Falini B., Kadin M.E. (1995)Blood 85(8): 2139-2146 and Terenzi A, Bolognesi A, Pasqualucci L, Flenghi L, Pileri S, Stein H, Kadin M, Bigerna B, Polito L, Tazzari PL, Martelli MF, Stirpe F, Falini B (1996) Br J Haematol 92(4): 872-879). The same approach is effective in patients with refractory Hodgkin's disease even if responses are short and partial.

Finally, an increase of the host defense against tumor can be accomplished by transfecting GITR or its ligand into the patient cell in vitro and subsequently reinfusing the transfected cells into the patient. A similar approach has been used with another system which is crucial in costimulation of T-cells (CD28/B7). Many tumors lack expression of B7-1 and this has been suggested to contribute to the failure of immune recognition of these diseases. In several murine models

transfection of CD28 ligands (B7-1 or B7-2) in the tumor cells causes the rejection of the tumor and the mice develop protective immunity against subsequent challenge with B7-1(-) (or B7-2(-)) untransfected tumors: (e.g. Matulonis U.A. et al. Blood (1995) 85(9); 2507-2515). However, in other models B7 transfection does not elicit rejection. Thus, transfection of other coaccessory molecules (such as GITR) may be useful.

SUMMARY OF THE INVENTION

The present invention discloses a novel member of the TNFR/NGFR family, designated GITR, and its splicing variants GITR-B and GITR-C.

In a first aspect, the present invention provides an isolated single or double stranded polynucleotide, typically DNA, having a nucleotide sequence which comprises: (a) a nucleotide sequence selected from the group consisting of (i) the sequence from nucleotide position 46 to nucleotide position 729 of SEQ ID NO. 1; (ii) the sequence from nucleotide position 46 to nucleotide position 930 of SEQ ID NO. 4; and (iii) the sequence from nucleotide position 46 to nucleotide position 714 of SEQ ID NO. 6; (b) sequences complementary to the sequences of (a); (c) sequences that, on expression, encode a polypeptide encoded by the sequences of (a); and (d) analogous sequences that hybridize under stringent conditions to the sequences of (a) or (b). A preferred embodiment is a DNA molecule. In another embodiment, the polynucleotide is an RNA molecule.

In another aspect, a DNA molecule of the present

invention is contained in an expression vector. The expression vector preferably further comprises an enhancer-promoter operatively linked to the polynucleotide. In a preferred embodiment, the DNA molecule in the vector is one of the sequences of SEQ ID NO. 1, SEQ ID NO. 4 and SEQ ID NO. 6.

The present invention still further provides for a host cell transformed with an expression vector of this invention. The host may be a prokaryotic or a eukaryotic cell. Example of a preferred prokaryotic host cell is *E. coli*, whereas, among the eukaryotic cells, preferred hosts are yeast or insect cells.

In a still further aspect the invention provides an isolated and purified polypeptide which is coded for by a nucleotide sequence selected from the group consisting of: (a) the sequence from nucleotide position 46 to nucleotide position 729 of SEQ ID NO. 1; the sequence from nucleotide position 46 to nucleotide position 930 of SEQ ID NO. 4; the sequence from nucleotide position 46 to nucleotide position 714 of SEQ ID NO. 6; (b) sequences complementary to the sequences of (a); (c) sequences that, on expression, encode a polypeptide encoded by the sequences of (a); and (d) analogous sequences that hybridize under stringent conditions to the sequences of (a) or (b).

Furthermore, the invention provides a recombinant process for the expression of a polypeptide according to the invention, which process comprises inserting a said polynucleotide of the invention into an appropriate expression vector, transfecting the expression vector into an appropriate host, growing the transfected host in

a suitable culture medium and purifying the said polypeptide from the culture medium.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides isolated and purified polynucleotides that encode the GITR receptor and its splicing variants, vectors containing these polynucleotides, host cells transformed with these vectors, a process of making the GITR receptor or its splicing variants using the above polynucleotides and vectors, and isolated and purified recombinant GITR receptor as well as its splicing variants.

For the purposes of the present invention as disclosed and claimed herein, the following is to be considered.
The amino acid sequences are presented in the amino to carboxy direction, from left to right. The amino and carboxy groups are not presented in the sequence.
The nucleotide sequences are presented by single strand only, in the 5' to 3' direction, from left to right.
Nucleotides and amino acids are represented in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by three letters code.

In one aspect, the present invention provides isolated and purified polynucleotides that encode the GITR receptor from mouse and its splicing variants. A polynucleotide of the present invention is an isolated single or double stranded polynucleotide having a nucleotide sequence which comprises:

(a) a nucleotide sequence selected from the group consisting of (i) the sequence from nucleotide position 46 to nucleotide position 729 of SEQ ID NO. 1; (ii) the sequence from nucleotide position 46 to nucleotide position 930 of SEQ ID NO. 4; and (iii) the sequence from nucleotide position 46 to nucleotide position 714 of SEQ ID NO. 6;

(b) sequences complementary to the sequences of (a);

(c) sequences that, on expression, encode a polypeptide encoded by the sequences of (a); and

(d) analogous sequences that hybridize under stringent conditions to the sequences of (a) or (b).

A polynucleotide of the invention may thus consist essentially of sequence (a), (b), (c) or (d). A preferred polynucleotide is a DNA molecule. In another embodiment, the polynucleotide is an RNA molecule.

The nucleotide sequences and deduced amino acid sequence of the mouse GITR gene and of its splicing variants herein disclosed are set forth in SEQ ID NOs. 1, 4 and 6. The nucleotide sequences of SEQ ID NOs. 1, 4 and 6 represent full length DNA clones of the sense strands of the mouse GITR gene and of its splicing variants GITR-B and GITR-C. All the isolated clones share a common extracytoplasmic sequence. In particular they have three cysteine pseudorepeats with the following structures:

1. the first pseudorepeat is similar to the first pseudorepeat of TNFRII (p75) which is considered to be the reference of the whole TNFR/NGFR family;

2. the second pseudorepeat is similar to the third pseudorepeat of TNFRII (p75); and

3. the third pseudorepeat is similar to the fourth

pseudorepeat of TNFRII (p75).

All the other members of the TNF/NGF Receptor family have a cysteine pseudorepeat similar to the second pseudorepeat of TNFRII, whereas GITR lacks that pseudorepeat. This second pseudorepeat of TNFRII is defined by the following motif: x-C-x(0,1)-[DEP]-x(2,3)-[FY]-x(6,9)-C-x(2)-[CH]-x(2,3)-C-x(8,11)-[CG]-x(7)-C-x.

The analysis of the GITR genomic DNA revealed that GITR RNA derives from the splicing of 5 exons. The boundary exon-intron and intron-exon are in agreement with the splicing rule. The start codon and the stop codon for GITR protein synthesis are located in the first exon and in the fifth exon, respectively. The fourth exon contains the sequence coding for the transmembrane domain, whereas the sequence coding for the cytoplasmic domain is contained in the fourth and the fifth exon.

From the analysis of numerous clones isolated with the library screening (performed to isolate full-length GITR) it was found that some of them resulted to be different from GITR. In fact, between exon 4 and exon 5, 11 bases more were present (belonging to the 3'end of intron 4) (see SEQ ID NO. 4). In other words, in this splicing, exon 5 is 11 bp longer (at the 5' end) than the exon 5 found in GITR. The protein putatively coded by this clone, called GITR-B, is different from GITR in the cytoplasmic domain (compare SEQ ID NOs. 2 and 5), due to the reading frame shift with respect to GITR. This quite long cytoplasmic domain does not have significant homology with other known proteins.

By performing RT-PCR, a new GITR splicing was observed (GITR-C, SEQ ID NO. 6). In GITR-C, the intron between

exon 4 and exon 5 is not spliced out. To confirm this finding, it was performed RT-PCR with other primers and it was demonstrated that GITR-C is another GITR splicing. The protein putatively coded by GITR-C is different from GITR and GITR-B in the cytoplasmic domain, due to the addition of 67 bp of intron 4 and a reading frame shift with respect to GITR and GITR-B (compare SEQ ID NOs. 2, 5 and 7). This cytoplasmic domain does not have significant homology with other known proteins.

The present invention also contemplates analogous DNA sequences which hybridize under stringent conditions to the DNA sequences set forth above. Stringent hybridization conditions are well known in the art and define a degree of sequence identity greater than about 80%, preferably greater than 90% or greater than 95%, to a sequence of the same length. The term "analogous" refers to those nucleotide sequences that encode analogous polypeptides, analogous polypeptides being those which have only conservative differences and which retain the characteristics and activities of GITR. An analogous polypeptide thus has three cysteine pseudorepeats, none of which is homologous to the second pseudorepeat of TNFRII. The three cysteine pseudorepeats of GITR which an analogous polypeptide can possess are described in more detail below in Example 2. No pseudorepeat corresponding to the second pseudorepeat of TNFRII is therefore present.

An analogous polypeptide may thus incorporate from 1 to 20, for example from 1 to 15 or from 1 to 10, such conservative substitutions. There may be 1, 2, 3, 4 or 5 conservative substitutions. The following Table 1 sets

out conservative substitutions which may be made. Amino acids in the same line may be substituted for each other:

Table 1 of conservative amino acid substitutions

Gly	Ala	
Val	Ile	Leu
Asp	Glu	
Asn	Gln	
Ser	Thr	
Lys	Arg	His
Phe	Tyr	

The present invention also contemplates naturally occurring allelic variations and mutations of the DNA sequences set forth above so long as those variations and mutations code, on expression, for a GITR receptor.

In particular, the present invention includes further splicing variants as defined above which can be identified with the aid of the information provided herein.

DNA sequences coding for a GITR receptor of any species of origin are also part of the present invention. Preferably the DNA sequences code for proteins of mammalian origin; more preferably the DNA sequences code for the mouse GITR protein.

As is well known in the art, because of the degeneracy of the genetic code, there are numerous other DNA molecules that can code for the same polypeptide as that encoded by the aforementioned nucleotide sequences of GITR and its splicing variants. The present invention, therefore,

contemplates those other DNA molecules which, on expression, encode the polypeptides of SEQ ID NOs. 2, 5 or 7. Having identified the amino acid residue sequence encoded by any of the above clones, and with knowledge of all triplet codons for each particular amino acid residue, it is possible to describe all such encoding DNA sequences. DNA molecules other than those specifically disclosed herein characterized simply by a change in a codon for a particular amino acid, are within the scope of this invention.

A table of amino acids and their representative abbreviations, symbols and codons is set forth below in the following Table 2.

Amino acid	Abbrev.	Symbol	Codon(s)					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	UGA	UGU				
Aspartic acid	Asp	D	GAC	GAU				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCA	CCC	CCG	CCU		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

As is well known in the art, codons constitute triplet sequences of nucleotides in mRNA molecules and, as such, are characterized by the base uracil (U) in place of base thymidine (T) (which is present in DNA molecules).

A simple change in a codon for the same amino acid residue within a polynucleotide will not change the structure of the encoded polypeptide.

The mouse GITR receptor of the present invention includes proteins homologous to, and having essentially the same biological properties as, the protein coded for by the nucleotide sequence herein disclosed. This definition is intended to encompass natural allelic variants of GITR sequence, in particular those deriving from the various splicing variants of the GITR receptor.

With the knowledge of the sequence information disclosed in the present invention, the expert in the art can identify and obtain DNA sequences which encode the GITR receptor from different sources (i.e. different tissues or different organisms) through a variety of means well known to him and disclosed by, for example, Maniatis et al., Molecular cloning: a laboratory manual, Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989).

For example, DNA which encodes the GITR receptor may be obtained by screening of cDNA or genomic DNA libraries with oligonucleotide probes generated from the GITR receptor gene sequence information provided herein. Probes may be labeled with a detectable group such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with known

procedures and used in conventional hybridization assays, as described by, for example, Maniatis et al. Molecular cloning: a laboratory manual, Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989).

The GITR gene sequence may alternatively be recovered by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers produced from the GITR receptor sequences provided herein. See U. S. Pat. Nos. 4,683,195 to Mullis et al. and U.S. Pat. No. 4,683,202 to Mullis. The PCR reaction provides a method for selectively increasing the concentration of a particular nucleic acid sequence even when that sequence has not been previously purified and is present only in a single copy in a particular sample. The method can be used to amplify either single- or double-stranded DNA. The essence of the method involves the use of two oligonucleotides probes to serve as primers for the template-dependent, polymerase mediated replication of a desired nucleic acid molecule.

The recombinant DNA molecules of the present invention can be produced through any of a variety of means well known to the experts in the art and disclosed by, for example, Maniatis et al. Molecular cloning: a laboratory manual, Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989).

In order to replicate the GITR receptor DNA sequence or the DNA sequence of its splicing variants, these must be cloned in an appropriate vector. A vector is a replicable DNA construct.

Vectors are used herein either to amplify DNA encoding the GITR receptor and/or to express DNA which encodes the

GITR receptor. An expression vector is a replicable DNA construct in which a DNA sequence encoding GITR receptor is operably linked to suitable control sequences capable of effecting the expression of the GITR receptor enzyme in a suitable host. DNA regions are operably linked when they are functionally related to each other. For example: a promoter is operably linked to a coding sequence if it controls the transcription of the sequence. Amplification vectors do not require expression control domains. All that is needed is the ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants.

DNA sequences encoding GITR receptor or its splicing variants may be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesiderable joining, and ligation with appropriate ligases. Techniques for such manipulation are disclosed by Maniatis et al. Molecular cloning: a laboratory manual, Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989) and are well known in the art.

Expression of the cloned sequence occurs when the expression vector is introduced into an appropriate host cell. If a prokaryotic expression vector is employed, then the appropriate host cell would be any prokaryotic cell capable of expressing the cloned sequences, for example *E. coli*. Similarly, if an eukaryotic expression

vector is employed, then the appropriate host cell would be any eukaryotic cell capable of expressing the cloned sequence. A yeast host may be employed, for example *S. cerevisiae*. Alternatively, insect cells may be used, in which case a baculovirus vector system may be appropriate. Another alternative host is a mammalian cell line, for example COS-1 cells.

The need for control sequences into the expression vector will vary depending upon the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding, for example a Shine-Dalgarno sequence, and sequences which control the termination of transcription and translation. Vectors useful for practising the present invention include plasmids, viruses (including phages), retroviruses, and integrable DNA fragments (i. e. fragments integrable into the host genome by homologous recombination). The vectors replicate and function independently of the host genome, or may, in some instances, integrate into the genome itself .

Expression vectors should contain a promoter which is recognized by the host organism. The promoter sequences of the present invention may be either prokaryotic, eukaryotic or viral. Example of suitable prokaryotic sequences include the P_R and P_L promoters of bacteriophage lambda (The bacteriophage Lambda, Hershey, A. D., Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1973); Lambda II, Hendrix, R. W., Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1980)); the trp, recA, heat

shock, and lacZ promoters of E. Coli and the SV40 early promoter (Benoist, C. et al. Nature 290: 304-310 (1981)).

As far as the Shine-Dalgarno sequence is concerned, preferred examples of suitable regulatory sequences are represented by the Shine-Dalgarno of the replicase gene of the phage MS-2 and of the gene cII of bacteriophage lambda. The Shine-Dalgarno sequence may be directly followed by the DNA encoding GITR receptor and result in the expression of the mature GITR protein.

Alternatively, the DNA encoding GITR may be preceded by a DNA sequence encoding a carrier peptide sequence. In this case, a fusion protein is produced in which the N-terminus of GITR is fused to a carrier peptide, which may help to increase the protein expression levels and intracellular stability, and provide simple means of purification. A preferred carrier peptide includes one or more of the IgG binding domains of protein A which are easily purified to homogeneity by affinity chromatography e. g. on IgG-coupled Sepharose. A DNA sequence encoding a recognition site for a proteolytic enzyme such as enterokinase, factor X or procollagenase may immediately precede the sequence for GITR to permit cleavage of the fusion protein to obtain the mature GITR protein.

Moreover, a suitable expression vector includes an appropriate marker which allows the screening of the transformed host cells. The transformation of the selected host is carried out using any one of the various techniques well known to the expert in the art and described in Maniatis et al. Molecular cloning: a laboratory manual, Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989).

One further embodiment of the invention is a prokaryotic host cell transformed with the said expression vector and able to produce, under appropriate culture conditions, the GITR receptor of the invention.

Cultures of cells derived from multicellular organisms are a desirable host for recombinant GITR synthesis. In principal, any eukaryotic cell culture is workable, whether from vertebrate or invertebrate cell culture, including insect cells. Propagation of such cells in cell culture has become a routine procedure. See Tissue Culture, Academic Press, Kruse and Patterson, eds. (1973). Examples of useful host cell lines are HeLa cells, CHO and COS cell lines. The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate and invertebrate cells are often provided by viral sources, for example, commonly used promoters are derived from Adenovirus 2, polyoma and SV40. See, e. g. U. S. Pat. No. 4,599, 308.

An origin of replication may be provided either by construction of the vector to include an exogenous origin or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter may be sufficient.

Rather than using vectors which contains viral origins of replication, one can transform mammalian cells by the method of cotransformation with a selectable marker and GITR DNA. An example of a suitable marker is dihydrofolate reductase (DHFR) or thymidine kinase. See U. S. Pat. No. 4,399,216.

Cloned genes and vectors of the present invention are useful to transform cells which do not ordinarly express

GITR to thereafter express this receptor. Such cells are useful as intermediates for making recombinant GITR preparations useful for drug screening.

Furthermore, structural data deriving from the analysis of the deduced amino acid sequences of the DNAs of the present invention are useful to design new drugs, more specific and therefore with a higher pharmacological potency.

Variants of the GITR receptor protein of the present invention (obtained as described above) could be present in different tissues and/or organs, and might represent potential new pharmacological targets to develop more specific drugs.

Cloned genes of the present invention , and oligonucleotides derived therefrom, are useful for screening for restriction fragment length polymorphism (RFLP) associated with certain disorders.

Oligonucleotides derived from the GITR DNA sequence or from the DNA sequences of its splicing variants disclosed in the present invention are useful as diagnostic tools for probing GITR gene expression in various tissues. For example, tissue can be probed *in situ* with oligonucleotide probes carrying detectable groups by conventional autoradiography techniques to investigate native expression of this receptor or pathological conditions relating thereto.

The present invention is explained in greater detail in the following examples. These examples are intended to be illustrative of the present invention, and should not be construed as limiting.

Example 1: Cloning of the GITR cDNA

In order to study the role of glucocorticoid hormones in the regulation of lymphocyte apoptosis, we undertook the isolation of mRNA induced by short-term (3h) and long term (24h) treatment with the synthetic glucocorticoid hormone dexamethasone (DEX, 10^{-7}M), in a spontaneously dividing $CD3^+$, $CD4^+$, $CD2^+$, $CD44^+$ line obtained by recloning the OVA-specific hybridoma T-cell line 3DO (Simonkevtz R., Kappler J., Marrak P. & Grey H. (1983) J. Exp. Med 158,303-309).

Cells were maintained in logarithmic growth in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 10nM Hepes and antibiotics RNA was isolated by using TRIzol LS reagent (GIBCO-BRL, Life Technologies, Paisley, Scotland). Briefly, 750 µl TRIzol LS were added to 250 µl medium containing $10\text{-}40\text{x}10^6$ cells. Following centrifugation, RNA was precipitated from the supernatant by isopropanol and the pellet was washed with 75% ethanol.

Comparing the cDNAs from untreated and DEX-treated (24h) cells by the differential display technique (Liang, P., & Pardee A.B. (1992) Science 257, 967-971), we identified some mRNAs detectable only in the treated cells.

Briefly, 0.1 µg DNA-free RNA were retrotranscribed (M-MLV reverse transcriptase from GIBCO-BRL) by using an anchored primer $T_{11}AC$. 40 Cycles of PCR were performed using $T_{11}AC$ and the OPA 5'CGCGGAGGTG3', SEQ ID NO:3. Three

independent samples of untreated 3DO cells were compared with 3 samples of 3h and 24h DEX-treated 3DO cells, by running a polyacrylamide gel. The radioactive bands present in each of the short- or long-term treated samples and absent in each of the untreated cells were cloned by using TA-cloning kit (Invitrogen) and considered for further research. The cloned DNA corresponding to GITR cDNA was about 400 bp long.

A library screening was performed in order to obtain the full length cDNA. A primary and secondary screening of a mouse T-cell (M30, CD4+) cDNA library (Stratagene) cloned unidirectionally in the Uni-ZAP XR vector was performed following the standard procedures (Sambrook K., Fritsch, E.F., & Maniatis, T. (1989) Molecular Cloning eds C. Nolan (Cold Spring Harbor Laboratory Press, New York)).

The 18 positive phages were *in vivo* excised through the ExAssist/SORL system, following the manufacturer's instructions. Positive bacterial clones were PCR screened and, from among the longest inserts, three were chosen for sequencing. The three clones had identical sequences.

The nucleotide sequence of GITR cDNA isolated by this procedure and the amino acid sequence encoded thereby are presented in SEQ ID NO: 1 and SEQ ID NO:2 respectively. GITR cDNA has a 684 bp open reading frame (ORF), beginning at nucleotide position 46 and extending to a TGA termination codon at position 730.

Three in-frame ATGs are found between position 46 and position 79. The first, at position 46, is surrounded by a sequence (AGCACTATGG) in good agreement with the consensus sequence for initiation of translation in eukaryotes (Kozak). The termination codon is followed by a 3' untranslated region of 276 bp. A canonical polyadenylation signal is present 18 bp before the poly-A tail.

Example 2: Characterization of the deduced GITR protein

The protein putatively coded by GITR mRNA is a cysteine-rich protein 228 amino acid long. Two hydrophobic regions were found, probably representing the signal peptide and a transmembrane domain. The site of cleavage of the signal peptide might be between Gly and Gln (amino acids 19 and 20 respectively in SEQ ID NOS: 1 and 2) despite the unusual presence of Asp at amino acid position 17 (SEQ ID NOS: 1 and 2). The transmembrane domain might span between position 154 and position 176. Based on these features, GITR can be classified as a type I transmembrane protein with 153 amino acids forming the extracellular domain and 52 amino acids forming the intracellular domain.

The molecular weight of the native protein calculated on the basis of the cDNA sequence is 25334 Da and this weight is consistent to that obtained after in vitro translation of the cloned GITR cDNA (see Example 3). The predicted molecular weight of the putative mature protein before further post-translational modifications is equal to 23321 Da and its isoelectric point is equal to 6.46.

The GITR amino acid sequence displays significant homologies with the 4-1BB receptor which belongs to the TNF/NGFR family. The extracellular domain of molecules belonging to the TNF/NGFR family is characterised by cysteine pseudorepeats whose functional properties have been defined. The canonical cysteine pseudo-repeat is formed by cysteine 1 (C1) that forms a disulfide bridge with cysteine 2 (C2), cysteine 3 (C3) that forms a disulfide bridge with cysteine 5 (C5) and cysteine 4 (C4) that forms a disulfide bridge with cysteine 6 (C6).

On the basis of the homology with the other TNF/NGFR members, three cysteine pseudorepeats can be identified in GITR similar to the structure of TNFR (p75) pseudorepeats 1, 3 and 4 respectively. The first pseudorepeat, from Cys at position 29 to Cys at position 60, although atypical, has some features similar to that of the first pseudorepeat of several proteins belonging to the TNF/NGFR family (CD30, CD27, TNFR p-55 and p-75, LTβR, Fas, NGFR, CD40, OX40). It is formed by C1, C2, C3 and C5. C6 is also present but, since C4 is not present, C6 should not form a disulfide bridge in this pseudorepeat.

The structure of the second pseudorepeat from Cys at position 62 to Cys at position 100 is similar to the third pseudorepeat of several proteins belonging to TNFR/NGFR family (TNFR p75, CD40, LTβR, CD30 and 4-1BB). It lacks C3 and C5 and has two cysteine residues which should form an extra disulfide bridge. The third cysteine pseudorepeat from Cys at position 103 to Cys at position

141 lacks C3 and C5 and shows extensive homologies with the pseudorepeat number 4 of several members of the TNFR/NGFR family (OX40, 4-1BB, CD40 and TNFR p75).

The cytoplasm domain of GITR has a high similarity with the intracellular domain of murine and human CD27 and 4-1BB (see Table 3), so that it could be hypothesized that 4-1BB, CD27 and GITR define a cytoplasmic domain ("life domain") of the TNFR/NGFR family which is different from the TNFR-Fas "death domain". Thus, GITR should activate intracellular pathways similar to those activated by CD27 and 4-1BB. We are the first to describe this domain since GITR is similar to both CD27 and 4-1BB, while CD27 and 4-1BB have a lower degree of similarity. The "life domain" should have a functional meaning since the similarity among the extracellular domain of GITR, CD27 and 4-1BB are much lower (at the same level of the other member of the family) and thus the common derivation from an ancestral gene can be excluded.

Table 3

CD27m	trm	..Q.RRNHG.PNEDRQ.....AVPEEPCPYSCPREEEGSAIPIQEDYR.KPEPAFYP
CD27h	trm	..Q.RR.KYGPNEDRQ.....AEPAEPCRYSCPREEEGSTIPIQEDYR.KPEPACSP
4-1BBm	trm	KWI.RK.KF.PHIFKQPFKKTTGAAQE.EDACSCRCPQEEEGG.GGGYEL
4-1BBh	trm	KRG.RK.KLLY.IFKQPFMRPVQTTQE.EDGCSCRFPEEEEGG....CEL
GITRm	trm	IWQLRRQHMCPRET.QPFAE.VQLSAE..DACSFQFPEEERGE...QTEE.KCHL.GGRWP
charge°		++ + +- + - -- ±--± -- + +
CONSENSUS^		....RR.K.....Q.....E.C....P.EE.G.....E..R.K...A..P
		.....K.H.....D.....K.H...G...
		..q.....p.e.r.pf.....ae.e.a.sy.c....e.....e.....
		.....k.....g..f.....d.....

° Charge of the amino acid residues present in at least 2 chains belonging to different receptors.

^ Amino acid residue identical of with similar function present in all the chains (capital letters) or in 3 or 4 chains (small letters).

Four potential glycosylation sites are present in the extracellular domain (amino acid positions 36, 40, 121 and 134). A potential phosphorylation site is present at position 199.

Example 3: In vitro translation of GITR cDNA.

Transcription/translation occurred from the T7 promoter using the Promega TNT kit. 949 bp DNA coding for GITR were cloned into pCR3 (Invitrogen) from which the portion coding for resistance to Geneticin had been removed. 1 µg of the resulting plasmid was added together with the translation system and 40 µCi [^{35}S]methionine (Amersham Life Science International) and translation was allowed to proceed for 90 minutes at 30°C according to manufacturer's instructions.

The product was analyzed by electrophoresis in 15% SDS-PAGE gels, followed by transfer to nitrocellulose (Bioblot NK, Costar) for 5 hours at 250 mA at 4°C in 25 mM Tris/glycine, pH 8.3, and 20% v/v methanol. After transfer the radioactive protein was revealed by autoradiography for 1 day. The molecular weight of the expressed product was consistent with the predicted molecular weight of 25334 Da.

Example 4: Tissue distribution and regulation of the expression

Northern blotting and PCR experiments demonstrated that mRNA expression of GITR was present at low level in the murine hybridoma T-cell line 3DO and was up-modulated in

cells treated with the synthetic glucocorticoid hormone dexamethasone (DEX). Modulation appeared 8 hours after DEX treatment but the effect was fully seen after 48 hours. A "run-on" experiment demonstrated a high increase of GITR transcription following 1 and 2 days of DEX treatment which could account for the increased mRNA concentration.

Lymphocytes from thymus, spleen and lymph nodes expressed low or undetectable mRNA levels of GITR as demonstrated by PCR or Northern blotting respectively. Treatment of lymphocytes from lymph nodes for 1-5 days with anti-CD3 antibodies, with ConA or TPA plus Ca-ionophore caused an up-modulation of GITR mRNA. Similar results were obtained with thymocytes and splenocytes.

Non-activated lymphocytes, and cells from the other non-lymphoid tissues evaluated, did not express GITR at levels which were detectable by competitive RT-PCR and Northern blotting.

Example 5: Preliminary evidence of GITR physiological role

Members of the TNF/NGFR family are involved in lymphocyte activation and are able to induce or inhibit cell death by apoptosis. In order to test the effects of GITR expression on apoptosis, we transfected cells of the hybridoma T cell line 3DO with an expression vector in which the GITR cDNA is expressed under the control of the CMV promoter. As controls, we also transfected cells with

the empty vector (clones pCR3/1-6) or the same vector expressing the same GITR sequence but in the anti-sense direction (RTIG, clones RTIG/1-6). After selection with Geneticin, cell clones were screened for GITR or RTIG expression by reverse-transcriptase PCR (RT-PCR). For each transfection, 6 clones were tested and used for functional characterization. In addition, 6 normal untransfected clones (nuc/1-6) were tested as a further control.

The parental plasmid used in the transfection experiments was pCR3 (Invitrogen). 979 bp DNA coding for GITR in the sense or antisense (RTIG) orientation was also cloned into a pCR3 plasmid from which the portion coding for resistance to Geneticin had been removed (pCR3/G-). 3DO cells were cotransfected with 5 µg of pCR3 and 15 µg of pCR3/G- to increase the amount of GITR or RTIG in the Geneticin resistant cells.

Plasmid preps were made with Qiagen's Maxiprep plasmid DNA kit. 3DO cells were electroporated at 300 mA, 960 µF in the presence of plasmid and cultured for 48h in the standard medium. Then Geneticin (0.5 mg/ml) was added to the cell culture and 200 µl of the cell suspension were plated in 96-wells plates (3 for each transfection). Following 10-15 days, no more than 15% of the wells presented alive growing cells. These cells were considered clones and PCR screened for the expression of exogenous GITR or RTIG. The six best clones were considered for functional studies.

Logarithmically growing cells were cultured in 96-well plates ($5x10^5$ cells/ml) coated overnight with anti-mouse CD3ϵ mAbs (Pharmingen, San Diego, CA)(10 µg/ml). 24 h later apoptosis was measured by flow cytometry as described (Migliorati G., Nicoletti I., Pagliacci M.C., D'Adamio L. & Riccardi C. (1993) Blood 81, 1352-1358) and as below specified. Briefly, after culturing, cells were centrifuged and the pellets were gently resuspended in 1.5 ml hypotonic PI solution (50 µg/ml in 0.1% sodium citrate plus 0.1% Triton X-100, Sigma, St. Louis, MO, USA). The tubes were kept at 4°C in the dark overnight.

The PI-fluorescence of individual nuclei was measured by flow cytometry with standard FACSCAN equipment (Becton Dickinson, Mountain View, USA). The nuclei traversed the light beam of a 488 nm Argon laser. A 560 nm dichroid mirror (DM 570) and a 600 nm band pass filter (band width 35 nm) were used to collect the red fluorescence due to PI DNA staining, and the data were recorded in logarithmic scale in a Hewlett Packard (HP 9000, model 310) computer. The percentage of apoptotic cell nuclei (sub-diploid DNA peak in the DNA fluorescence histogram) was calculated with specific FACSCAN research software (Lysis II, Becton Dickinson).

The results, summerised in Table 4 below, showed that cell clones overexpressing GITR (clones GITR-6) were all variably resistant to anti-CD3 mAb-induced apoptosis (apoptosis between 5 and 10% as compared to 50-60% of pCR3 control clones: pCR3/1-6; $P<0.01$). On the contrary, clones expressing antisense RTIG RNA (clones RTIG/1-6)

were more sensitive to anti-CD3-induced apoptosis (apoptosis between 80 and 93% as compared to 50-60% of pCR3 control clones; $P<0.01$) suggesting that antisense expression may have inhibited the low levels of endogenous RTIG expression. No significant differences between pCR3 clones and normal untransfected clones (nuc/1-6: apoptosis between 45 and 55%, with $P>0.05$ comparing pCR3 clones with nuc) were detectable.

These results suggest that GITR can modulate T cell apoptosis triggered by T-cell receptor (TCR)/CD3 complex.

Table 4

Clones	Treated	Untreated
nuc/1	6	48
nuc/2	4	45
nuc/3	3	53
nuc/4	2	50
nuc/5	8	49
nuc/6	4	55
pCR3/1	5	56
pCR3/2	3	50
pCR3/3	5	60
pCR3/4	5	54
pCR3/5	3	55
pCR3/6	5	57
GITR/1	6	7
GITR/2	5	11
GITR/3	10	19
GITR/4	5	2

GITR/5	5	4
GITR/6	6	13
RTIG/1	12	93
RTIG/2	3	90
RTIG/3	4	91
RTIG/4	11	91
RTIG/5	5	80
RTIG/6	3	93

nuc = normal untransfected clones (first control)

pCR3 = empty vector transfected clones (second control)

GITR = sense GITR transfected clones

RTIG = antisense GITR transfected clones

Example 6: GITR-B cloning by library screening

A primary and secondary screening of a mouse T-cell (M30, CD4+) cDNA library (Stratagene, La Jolla, CA) cloned unidirectionally in the Uni-ZAP XR vector was performed following the standard procedures. The 18 positive phages were *in vivo* excised through the ExAssist/SORL system, following the manufacturer's instructions. Positive bacterial clones were PCR screened and most of them were sequenced. Three of them resulted to be different from GITR. In fact, between exon 4 and exon 5, 11 bases more were present (belonging to the 3'end of intron 4). In other words, in this splicing, exon 5 is 11 bp longer (at the 5' end) than the exon 5 found in GITR.

Example 7: GITR-C cloning by RT-PCR

RNA was isolated by using the TRIzol LS reagent (GIBCO-BRL, Life Technologies, Paisley, Scotland) following the manufacturer instruction and treated with DNAse RNAse-free (Promega). For the reverse transcriptase reaction (4h at 37°C), 1 µg RNA and 1 µl AMV reverse transcriptase (Promega) were used. Then 0.6 ml of the product reaction were used for the PCR (final volume of 20 µl) together with the standard reagents and 0.1 ml Taq Gold (Perkin Elmer Corporation, Nolwalk, Connecticut). DNA oligonucleotide primers were synthesized in an Oligo-1000 DNA synthesizer (Beckman, Fullerton, CA).

GITR-C was obtained with several primers located on exon 4 (forward) and on exon 5 (reverse). However, the product obtained could derive by a contaminating DNA (despite DNAse treatment), since the sequence obtained was identical to the genomic sequence of GITR. To demonstrate that GITR-C derived by a cDNA (and thus a mRNA), an RT-PCR was performed by using a forward primer located on exon 2 (5'ccaggccagagggtggagt3') (SEQ ID NO. 8) and a reverse primer located on the boundary intron 4-exon 5 (5'gaatggctgggtctctgtagta3') (SEQ ID NO. 9). Upon cloning and sequencing the RT-PCR product resulted to derive by an RNA splicing which assembles exons 2, 3 and 4, the intron 4 and exon 5, thus confirming that GITR-C derived by an mRNA.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: PHARMACIA & UPJOHN S.p.A.
(B) STREET: Via Robert Koch 1.2
(C) CITY: Milan
(E) COUNTRY: Italy
(F) POSTAL CODE (ZIP): 20152
(G) TELEPHONE: +39-2-48381
(H) TELEFAX: +39-2-48385397

(ii) TITLE OF INVENTION: Receptor belonging to the TNF/NGF receptor family

(iii) NUMBER OF SEQUENCES: 9

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1020 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:46..729

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CGGGAACTCC TGAAATCAGC CGACAGAAGA CTCAGGAGAA GCACT ATG GGG GCA 54
Met Gly Ala
1

```
TGG GCC ATG CTG TAT GGA GTC TCG ATG CTC TGT GTG CTG GAC CTA GGT      102
Trp Ala Met Leu Tyr Gly Val Ser Met Leu Cys Val Leu Asp Leu Gly
  5                  10                  15

CAG CCG AGT GTA GTT GAG GAG CCT GGC TGT GGC CCT GGC AAG GTT CAG      150
Gln Pro Ser Val Val Glu Glu Pro Gly Cys Gly Pro Gly Lys Val Gln
 20                  25                  30                  35

AAC GGA AGT GGC AAC AAC ACT CGC TGC TGC AGC CTG TAT GCT CCA GGC      198
Asn Gly Ser Gly Asn Asn Thr Arg Cys Cys Ser Leu Tyr Ala Pro Gly
                 40                  45                  50

AAG GAG GAC TGT CCA AAA GAA AGG TGC ATA TGT GTC ACA CCT GAG TAC      246
Lys Glu Asp Cys Pro Lys Glu Arg Cys Ile Cys Val Thr Pro Glu Tyr
             55                  60                  65

CAC TGT GGA GAC CCT CAG TGC AAG ATC TGC AAG CAC TAC CCC TGC CAA      294
His Cys Gly Asp Pro Gln Cys Lys Ile Cys Lys His Tyr Pro Cys Gln
         70                  75                  80

CCA GGC CAG AGG GTG GAG TCT CAA GGG GAT ATT GTG TTT GGC TTC CGG      342
Pro Gly Gln Arg Val Glu Ser Gln Gly Asp Ile Val Phe Gly Phe Arg
  85                  90                  95

TGT GTT GCC TGT GCC ATG GGC ACC TTC TCC GCA GGT CGT GAC GGT CAC      390
Cys Val Ala Cys Ala Met Gly Thr Phe Ser Ala Gly Arg Asp Gly His
100                 105                 110                 115

TGC AGA CTT TGG ACC AAC TGT TCT CAG TTT GGA TTT CTC ACC ATG TTC      438
Cys Arg Leu Trp Thr Asn Cys Ser Gln Phe Gly Phe Leu Thr Met Phe
                120                 125                 130

CCT GGG AAC AAG ACC CAC AAT GCT GTG TGC ATC CCG GAG CCA CTG CCC      486
Pro Gly Asn Lys Thr His Asn Ala Val Cys Ile Pro Glu Pro Leu Pro
            135                 140                 145

ACT GAG CAA TAC GGC CAT TTG ACT GTC ATC TTC CTG GTC ATG GCT GCA      534
Thr Glu Gln Tyr Gly His Leu Thr Val Ile Phe Leu Val Met Ala Ala
        150                 155                 160

TGC ATT TTC TTC CTA ACC ACA GTC CAG CTC GGC CTG CAC ATA TGG CAG      582
Cys Ile Phe Phe Leu Thr Thr Val Gln Leu Gly Leu His Ile Trp Gln
    165                 170                 175

CTG AGG AGG CAA CAC ATG TGT CCT CGA GAG ACC CAG CCA TTC GCG GAG      630
Leu Arg Arg Gln His Met Cys Pro Arg Glu Thr Gln Pro Phe Ala Glu
180                 185                 190                 195
```

```
GTG CAG TTG TCA GCT GAG GAT GCT TGC AGC TTC CAG TTC CCT GAG GAG      678
Val Gln Leu Ser Ala Glu Asp Ala Cys Ser Phe Gln Phe Pro Glu Glu
                200                 205                 210

GAA CGC GGG GAG CAG ACA GAA GAA AAG TGT CAT CTG GGG GGT CGG TGG      726
Glu Arg Gly Glu Gln Thr Glu Glu Lys Cys His Leu Gly Gly Arg Trp
            215                 220                 225

CCA TGAGGCCTGG TCTTCCTCTG TGCCCCAAGC CAGACGCTAC AAGACTTGCC           779
Pro

CAGCTATACC CTTGGTGAGA GCAGGGGCCA TGCTCTGCAC CCTTCCCTGG GCCTGGCCCT    839

GCTCCCCTCA ACAGTGGCGG AAGTGGGTGT ATGAGAGCGG TGAGTTACGA TTGGGCCCTA    899

TGGCTGCCTT TCTCATTTGA CAGCTCTGTT GGAGTAGGGT CTTTGGGCCC ACCAAGAGCA    959

CCACGTTTAG CACAAGATCT TGTACAAGAA TAAATACTTG TCTAGTAAAA AAAAAAAAAA    1019

A                                                                    1020
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 228 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Gly Ala Trp Ala Met Leu Tyr Gly Val Ser Met Leu Cys Val Leu
 1               5                  10                  15

Asp Leu Gly Gln Pro Ser Val Val Glu Glu Pro Gly Cys Gly Pro Gly
            20                  25                  30

Lys Val Gln Asn Gly Ser Gly Asn Asn Thr Arg Cys Cys Ser Leu Tyr
        35                  40                  45

Ala Pro Gly Lys Glu Asp Cys Pro Lys Glu Arg Cys Ile Cys Val Thr
    50                  55                  60

Pro Glu Tyr His Cys Gly Asp Pro Gln Cys Lys Ile Cys Lys His Tyr
65                  70                  75                  80
```

Pro Cys Gln Pro Gly Gln Arg Val Glu Ser Gln Gly Asp Ile Val Phe
85 90 95

Gly Phe Arg Cys Val Ala Cys Ala Met Gly Thr Phe Ser Ala Gly Arg
100 105 110

Asp Gly His Cys Arg Leu Trp Thr Asn Cys Ser Gln Phe Gly Phe Leu
115 120 125

Thr Met Phe Pro Gly Asn Lys Thr His Asn Ala Val Cys Ile Pro Glu
130 135 140

Pro Leu Pro Thr Glu Gln Tyr Gly His Leu Thr Val Ile Phe Leu Val
145 150 155 160

Met Ala Ala Cys Ile Phe Phe Leu Thr Thr Val Gln Leu Gly Leu His
165 170 175

Ile Trp Gln Leu Arg Arg Gln His Met Cys Pro Arg Glu Thr Gln Pro
180 185 190

Phe Ala Glu Val Gln Leu Ser Ala Glu Asp Ala Cys Ser Phe Gln Phe
195 200 205

Pro Glu Glu Glu Arg Gly Glu Gln Thr Glu Glu Lys Cys His Leu Gly
210 215 220

Gly Arg Trp Pro
225

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CGCGGAGGTG 10

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1031 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:46..930

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
CGGGAACTCC TGAAATCAGC CGACAGAAGA CTCAGGAGAA GCACT ATG GGG GCA          54
                                                  Met Gly Ala
                                                    1

TGG GCC ATG CTG TAT GGA GTC TCG ATG CTC TGT GTG CTG GAC CTA GGT        102
Trp Ala Met Leu Tyr Gly Val Ser Met Leu Cys Val Leu Asp Leu Gly
  5                  10                  15

CAG CCG AGT GTA GTT GAG GAG CCT GGC TGT GGC CCT GGC AAG GTT CAG        150
Gln Pro Ser Val Val Glu Glu Pro Gly Cys Gly Pro Gly Lys Val Gln
 20                  25                  30                  35

AAC GGA AGT GGC AAC AAC ACT CGC TGC TGC AGC CTG TAT GCT CCA GGC        198
Asn Gly Ser Gly Asn Asn Thr Arg Cys Cys Ser Leu Tyr Ala Pro Gly
                 40                  45                  50

AAG GAG GAC TGT CCA AAA GAA AGG TGC ATA TGT GTC ACA CCT GAG TAC        246
Lys Glu Asp Cys Pro Lys Glu Arg Cys Ile Cys Val Thr Pro Glu Tyr
             55                  60                  65

CAC TGT GGA GAC CCT CAG TGC AAG ATC TGC AAG CAC TAC CCC TGC CAA        294
His Cys Gly Asp Pro Gln Cys Lys Ile Cys Lys His Tyr Pro Cys Gln
         70                  75                  80

CCA GGC CAG AGG GTG GAG TCT CAA GGG GAT ATT GTG TTT GGC TTC CGG        342
Pro Gly Gln Arg Val Glu Ser Gln Gly Asp Ile Val Phe Gly Phe Arg
  85                  90                  95
```

```
TGT GTT GCC TGT GCC ATG GGC ACC TTC TCC GCA GGT CGT GAC GGT CAC      390
Cys Val Ala Cys Ala Met Gly Thr Phe Ser Ala Gly Arg Asp Gly His
100                 105                 110                 115

TGC AGA CTT TGG ACC AAC TGT TCT CAG TTT GGA TTT CTC ACC ATG TTC      438
Cys Arg Leu Trp Thr Asn Cys Ser Gln Phe Gly Phe Leu Thr Met Phe
                120                 125                 130

CCT GGG AAC AAG ACC CAC AAT GCT GTG TGC ATC CCG GAG CCA CTG CCC      486
Pro Gly Asn Lys Thr His Asn Ala Val Cys Ile Pro Glu Pro Leu Pro
            135                 140                 145

ACT GAG CAA TAC GGC CAT TTG ACT GTC ATC TTC CTG GTC ATG GCT GCA      534
Thr Glu Gln Tyr Gly His Leu Thr Val Ile Phe Leu Val Met Ala Ala
        150                 155                 160

TGC ATT TTC TTC CTA ACC ACA GTC CAG CTC GGC CTG CAC ATA TGG CAG      582
Cys Ile Phe Phe Leu Thr Thr Val Gln Leu Gly Leu His Ile Trp Gln
    165                 170                 175

CTG AGG AGG CAA CAC ATG TGT CCC CGA GTT TTA CTA CAG AGA CCC AGC      630
Leu Arg Arg Gln His Met Cys Pro Arg Val Leu Leu Gln Arg Pro Ser
180                 185                 190                 195

CAT TCG CGG AGG TGC AGT TGT CAG CTG AGG ATG CTT GCA GCT TCC AGT      678
His Ser Arg Arg Cys Ser Cys Gln Leu Arg Met Leu Ala Ala Ser Ser
                200                 205                 210

TCC CTG AGG AGG AAC GCG GGG AGC AGA CAG AAG AAA AGT GTC ATC TGG      726
Ser Leu Arg Arg Asn Ala Gly Ser Arg Gln Lys Lys Ser Val Ile Trp
            215                 220                 225

GGG GTC GGT GGC CAT GAG GCC TGG TCT TCC TCT GTG CCC CAA GCC AGA      774
Gly Val Gly Gly His Glu Ala Trp Ser Ser Ser Val Pro Gln Ala Arg
        230                 235                 240

CGC TAC AAG ACT TGC CCA GCT ATA CCC TTG GTG AGA GCA GGG GCC ATG      822
Arg Tyr Lys Thr Cys Pro Ala Ile Pro Leu Val Arg Ala Gly Ala Met
    245                 250                 255

CTC TGC ACC CTT CCC TGG GCC TGG CCC TGC TCC CCT CAA CAG TGG CGG      870
Leu Cys Thr Leu Pro Trp Ala Trp Pro Cys Ser Pro Gln Gln Trp Arg
260                 265                 270                 275

AAG TGG GTG TAT GAG AGC GGT GAG TTA CGA TTG GGC CCT ATG GCT GCC      918
Lys Trp Val Tyr Glu Ser Gly Glu Leu Arg Leu Gly Pro Met Ala Ala
                280                 285                 290
```

TTT CTC ATT TGA CAGCTCTGTT GGAGTAGGGT CTTTGGGCCC ACCAAGAGCA 970
Phe Leu Ile *

CCACGTTTAG CACAAGATCT TGTACAAGAA TAAATACTTG TCTAGTAAAA AAAAAAAAAA 1030

A 1031

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 295 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Gly Ala Trp Ala Met Leu Tyr Gly Val Ser Met Leu Cys Val Leu
1 5 10 15

Asp Leu Gly Gln Pro Ser Val Val Glu Glu Pro Gly Cys Gly Pro Gly
20 25 30

Lys Val Gln Asn Gly Ser Gly Asn Asn Thr Arg Cys Cys Ser Leu Tyr
35 40 45

Ala Pro Gly Lys Glu Asp Cys Pro Lys Glu Arg Cys Ile Cys Val Thr
50 55 60

Pro Glu Tyr His Cys Gly Asp Pro Gln Cys Lys Ile Cys Lys His Tyr
65 70 75 80

Pro Cys Gln Pro Gly Gln Arg Val Glu Ser Gln Gly Asp Ile Val Phe
85 90 95

Gly Phe Arg Cys Val Ala Cys Ala Met Gly Thr Phe Ser Ala Gly Arg
100 105 110

Asp Gly His Cys Arg Leu Trp Thr Asn Cys Ser Gln Phe Gly Phe Leu
115 120 125

Thr Met Phe Pro Gly Asn Lys Thr His Asn Ala Val Cys Ile Pro Glu
130 135 140

```
Pro Leu Pro Thr Glu Gln Tyr Gly His Leu Thr Val Ile Phe Leu Val
145                 150                 155                 160

Met Ala Ala Cys Ile Phe Phe Leu Thr Thr Val Gln Leu Gly Leu His
                165                 170                 175

Ile Trp Gln Leu Arg Arg Gln His Met Cys Pro Arg Val Leu Leu Gln
            180                 185                 190

Arg Pro Ser His Ser Arg Arg Cys Ser Cys Gln Leu Arg Met Leu Ala
        195                 200                 205

Ala Ser Ser Ser Leu Arg Arg Asn Ala Gly Ser Arg Gln Lys Lys Ser
    210                 215                 220

Val Ile Trp Gly Val Gly Gly His Glu Ala Trp Ser Ser Ser Val Pro
225                 230                 235                 240

Gln Ala Arg Arg Tyr Lys Thr Cys Pro Ala Ile Pro Leu Val Arg Ala
                245                 250                 255

Gly Ala Met Leu Cys Thr Leu Pro Trp Ala Trp Pro Cys Ser Pro Gln
            260                 265                 270

Gln Trp Arg Lys Trp Val Tyr Glu Ser Gly Glu Leu Arg Leu Gly Pro
        275                 280                 285

Met Ala Ala Phe Leu Ile  *
    290                 295
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1087 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:46..714

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
CGGGAACTCC TGAAATCAGC CGACAGAAGA CTCAGGAGAA GCACT ATG GGG GCA          54
                                                  Met Gly Ala
                                                    1

TGG GCC ATG CTG TAT GGA GTC TCG ATG CTC TGT GTG CTG GAC CTA GGT       102
Trp Ala Met Leu Tyr Gly Val Ser Met Leu Cys Val Leu Asp Leu Gly
  5                  10                  15

CAG CCG AGT GTA GTT GAG GAG CCT GGC TGT GGC CCT GGC AAG GTT CAG       150
Gln Pro Ser Val Val Glu Glu Pro Gly Cys Gly Pro Gly Lys Val Gln
 20                  25                  30                  35

AAC GGA AGT GGC AAC AAC ACT CGC TGC TGC AGC CTG TAT GCT CCA GGC       198
Asn Gly Ser Gly Asn Asn Thr Arg Cys Cys Ser Leu Tyr Ala Pro Gly
                 40                  45                  50

AAG GAG GAC TGT CCA AAA GAA AGG TGC ATA TGT GTC ACA CCT GAG TAC       246
Lys Glu Asp Cys Pro Lys Glu Arg Cys Ile Cys Val Thr Pro Glu Tyr
            55                  60                  65

CAC TGT GGA GAC CCT CAG TGC AAG ATC TGC AAG CAC TAC CCC TGC CAA       294
His Cys Gly Asp Pro Gln Cys Lys Ile Cys Lys His Tyr Pro Cys Gln
        70                  75                  80

CCA GGC CAG AGG GTG GAG TCT CAA GGG GAT ATT GTG TTT GGC TTC CGG       342
Pro Gly Gln Arg Val Glu Ser Gln Gly Asp Ile Val Phe Gly Phe Arg
  85                  90                  95

TGT GTT GCC TGT GCC ATG GGC ACC TTC TCC GCA GGT CGT GAC GGT CAC       390
Cys Val Ala Cys Ala Met Gly Thr Phe Ser Ala Gly Arg Asp Gly His
100                 105                 110                 115

TGC AGA CTT TGG ACC AAC TGT TCT CAG TTT GGA TTT CTC ACC ATG TTC       438
Cys Arg Leu Trp Thr Asn Cys Ser Gln Phe Gly Phe Leu Thr Met Phe
                120                 125                 130

CCT GGG AAC AAG ACC CAC AAT GCT GTG TGC ATC CCG GAG CCA CTG CCC       486
Pro Gly Asn Lys Thr His Asn Ala Val Cys Ile Pro Glu Pro Leu Pro
            135                 140                 145

ACT GAG CAA TAC GGC CAT TTG ACT GTC ATC TTC CTG GTC ATG GCT GCA       534
Thr Glu Gln Tyr Gly His Leu Thr Val Ile Phe Leu Val Met Ala Ala
        150                 155                 160
```

```
TGC ATT TTC TTC CTA ACC ACA GTC CAG CTC GGC CTG CAC ATA TGG CAG        582
Cys Ile Phe Phe Leu Thr Thr Val Gln Leu Gly Leu His Ile Trp Gln
    165                 170                 175

CTG AGG AGG CAA CAC ATG TGT CCC CGA GGT CAG TTG TGT CCC AGG GAA        630
Leu Arg Arg Gln His Met Cys Pro Arg Gly Gln Leu Cys Pro Arg Glu
180                 185                 190                 195

GGG GAA AAT GTG TCT CAG GCC CCT CAC TTA CCG CAG TTT TAC TAC AGA        678
Gly Glu Asn Val Ser Gln Ala Pro His Leu Pro Gln Phe Tyr Tyr Arg
                200                 205                 210

GAC CCA GCC ATT CGC GGA GGT GCA GTT GTC AGC TGA GGATGCTTGC             724
Asp Pro Ala Ile Arg Gly Gly Ala Val Val Ser  *
            215                 220

AGCTTCCAGT TCCCTGAGGA GGAACGCGGG GAGCAGACAG AAGAAAAGTG TCATCTGGGG      784

GGTCGGTGGC CATGAGGCCT GGTCTTCCTC TGTGCCCCAA GCCAGACGCT ACAAGACTTG      844

CCCAGCTATA CCCTTGGTGA GAGCAGGGGC CATGCTCTGC ACCCTTCCCT GGGCCTGGCC      904

CTGCTCCCCT CAACAGTGGC GGAAGTGGGT GTATGAGAGC GGTGAGTTAC GATTGGGCCC      964

TATGGCTGCC TTTCTCATTT GACAGCTCTG TTGGAGTAGG GTCTTTGGGC CCACCAAGAG     1024

CACCACGTTT AGCACAAGAT CTTGTACAAG AATAAATACT TGTCTAGTAA AAAAAAAAAA     1084

AAA                                                                   1087
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 223 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Met Gly Ala Trp Ala Met Leu Tyr Gly Val Ser Met Leu Cys Val Leu
  1               5                  10                  15

Asp Leu Gly Gln Pro Ser Val Val Glu Glu Pro Gly Cys Gly Pro Gly
             20                  25                  30
```

```
Lys Val Gln Asn Gly Ser Gly Asn Asn Thr Arg Cys Cys Ser Leu Tyr
        35                  40                  45

Ala Pro Gly Lys Glu Asp Cys Pro Lys Glu Arg Cys Ile Cys Val Thr
    50                  55                  60

Pro Glu Tyr His Cys Gly Asp Pro Gln Cys Lys Ile Cys Lys His Tyr
65                  70                  75                  80

Pro Cys Gln Pro Gly Gln Arg Val Glu Ser Gln Gly Asp Ile Val Phe
                85                  90                  95

Gly Phe Arg Cys Val Ala Cys Ala Met Gly Thr Phe Ser Ala Gly Arg
            100                 105                 110

Asp Gly His Cys Arg Leu Trp Thr Asn Cys Ser Gln Phe Gly Phe Leu
        115                 120                 125


Thr Met Phe Pro Gly Asn Lys Thr His Asn Ala Val Cys Ile Pro Glu
    130                 135                 140

Pro Leu Pro Thr Glu Gln Tyr Gly His Leu Thr Val Ile Phe Leu Val
145                 150                 155                 160

Met Ala Ala Cys Ile Phe Phe Leu Thr Thr Val Gln Leu Gly Leu His
                165                 170                 175

Ile Trp Gln Leu Arg Arg Gln His Met Cys Pro Arg Gly Gln Leu Cys
            180                 185                 190

Pro Arg Glu Gly Glu Asn Val Ser Gln Ala Pro His Leu Pro Gln Phe
        195                 200                 205

Tyr Tyr Arg Asp Pro Ala Ile Arg Gly Gly Ala Val Val Ser  *
    210                 215                 220
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CCAGGCCAGA GGGTGGAGT 19

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GAATGGCTGG GTCTCTGTAG TA 22

CLAIMS

1. An isolated single or double stranded polynucleotide having a nucleotide sequence which comprises:

(a) a nucleotide sequence selected from the group consisting of (i) the sequence from nucleotide position 46 to nucleotide position 729 of SEQ ID NO. 1; (ii) the sequence from nucleotide position 46 to nucleotide position 930 of SEQ ID NO. 4; and (iii) the sequence from nucleotide position 46 to nucleotide position 714 of SEQ ID NO. 6;

(b) sequences complementary to the sequences of (a);

(c) sequences that, on expression, encode a polypeptide encoded by the sequences of (a); and

(d) analogous sequences that hybridize under stringent conditions to the sequences of (a) or (b).

2. The polynucleotide of claim 1 that is a DNA molecule.

3. The polynucleotide of claim 1 that is a RNA molecule.

4. The polynucleotide of claim 2 wherein the nucleotide sequence is any one of the nucleotide sequences of (a).

5. A vector comprising a polynucleotide as defined in any one of claims 1 to 4.

6. A vector accoding to claim 5, which vector is a plasmid.

7. A vector according to claim 5, which vector is a virus.

8. A host cell transformed with a vector according to claim 5.

9. A transformed host cell according to claim 8, which cell is a bacterial cell.

10. A transformed host cell according to claim 8, which cell is a yeast cell.

11. A transformed host cell according to claim 8, which cell is an insect cell.

12. An isolated and purified polypeptide which is coded for by a polynucleotide according to any one of claims 1 to 4.

13. A polypeptide according to claim 12 which has the amino acid sequence shown in SEQ ID NO.2.

14. A polypeptide according to claim 12 which has the amino acid sequence shown in SEQ ID NO.5.

15. A polypeptide according to claim 12 which has the amino acid sequence shown in SEQ ID NO.7.

16. A recombinant process for the expression of a polypeptide which is coded for by a polynucleotide according to any one of claims 1 to 4, which process

comprises inserting a said polynucleotide into an appropriate expression vector, transfecting the expression vector into an appropriate host, growing the transfected host in a suitable culture medium and purifying the said polypeptide from the culture medium.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/06252

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705 C12Q1/68 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FIORUCCI S ET AL: "Isolated guinea pig gastric chief cells express tumour necrosis factor receptors coupled with the sphingomyelin pathway." GUT, (1996 FEB) 38 (2) 182-9. JOURNAL CODE: FVT. ISSN: 0017-5749., XP002061243 see the whole document ---	1-16
P,X	NOCENTINI G ET AL: "A new member of the tumor necrosis factor/nerve growth factor receptor family inhibits T cell receptor-induced apoptosis." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 JUN 10) 94 (12) 6216-21. JOURNAL CODE: PV3. ISSN: 0027-8424., XP002061244 see the whole document --- -/--	1-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
3 April 1998	20/04/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Oderwald, H

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 97/06252

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
E	WO 98 06842 A (SCHERING CORP) 19 February 1998 see abstract; examples 1,3,4,6 see the claims	1-16

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter. nal Application No
PCT/EP 97/06252

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9806842 A	19-02-98	NONE	